Immunity, Vol. 7, 691–701, November, 1997, Copyright ©1997 by Cell Press

An Indirect Effect of Stat5a in IL-2–Induced Proliferation: A Critical Role for Stat5a in IL-2–Mediated IL-2 Receptor α Chain Induction

Hiroshi Nakajima,* Xiu-Wen Liu,† Anthony Wynshaw-Boris,‡ Louis A. Rosenthal,§ Kazunori Imada,* David S. Finbloom,§ Lothar Hennighausen,† and Warren J. Leonard*‖

*Laboratory of Molecular Immunology
National Heart, Lung, and Blood Institute
†Laboratory of Biochemistry and Metabolism
National Institute of Diabetes
and Digestive and Kidney Diseases
‡Laboratory of Genetic Disease Research
National Human Genome Research Institute
National Institutes of Health
Bethesda, Maryland 20892
§Division of Cytokine Biology
Center for Biologics Evaluation and Research
Food and Drug Administration
Bethesda, Maryland 20892

Summary

Stat5a was identified as a prolactin-induced transcription factor but also is activated by other cytokines, including interleukin-2 (IL-2) and IL-7. We have now analyzed the immune system of Stat5a-deficient mice. Stat5a$^{-/-}$ splenocytes exhibited defective IL-2–induced expression of the IL-2 receptor α chain (IL-2Rα), a protein that together with IL-2Rβ and the common cytokine receptor γ chain (γ_c) mediates high-affinity IL-2 binding. Correspondingly, Stat5a$^{-/-}$ splenocytes exhibited markedly decreased proliferation to IL-2, although maximal proliferation was still achieved at IL-2 concentrations high enough to titrate intermediate-affinity IL-2Rβ/γ_c receptors. Thus, defective Stat5a expression results in diminished proliferation by an indirect mechanism, resulting from defective receptor expression. Correspondingly, we show that Stat5a is essential for maximal responsiveness to antigenic stimuli in vivo, underscoring the physiological importance of IL-2–induced IL-2Rα expression.

Introduction

Signal transducers and activators of transcription (STAT) proteins are cytosolic latent transcription factors that are rapidly activated following cellular exposure to interferons, cytokines, or growth factors (reviewed by Schindler and Darnell, 1995; Ihle, 1996; Horvath and Darnell, 1997; Leonard and O'Shea, 1998). A total of seven different STAT proteins have been identified. Much investigation has centered on the degree of specificity for each STAT protein and the extent to which STATs contribute to proliferation versus differentiation. At least some STATs exhibit selective actions for one or a limited number of cytokines (reviewed by Leonard, 1996; Horvath and Darnell, 1997). For example, in mice in which Stat1 (Durbin et al., 1996; Meraz et al., 1996), Stat4 (Kaplan et al., 1996b; Thierfelder et al., 1996), or Stat6 (Kaplan et al., 1996a; Shimoda et al., 1996; Takeda et al., 1996) was separately disrupted by homologous recombination, relatively specific defects were found corresponding to defective signaling in response to interferons, interleukin-12 (IL-12), and IL-4/IL-13, respectively, even though in the case of Stat1, a large number of cytokines and growth factors have been reported to activate this STAT protein.

Stat5 was originally identified as a mammary gland factor induced by prolactin (Wakao et al., 1994). Subsequently, this protein was renamed Stat5a when a second, homologous gene, denoted Stat5b, was identified (Azam et al., 1995; Liu et al., 1995; Mui et al., 1995b; Lin et al., 1996). Both Stat5a and Stat5b are activated not only by prolactin but also by a wide range of other cytokines, including growth hormone, erythropoietin, thrombopoietin, IL-3, granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-5, IL-2, IL-7, IL-9, and IL-15 (Schindler and Darnell, 1995). Nevertheless, the specificities of their actions are demonstrated by the observations that Stat5a-deficient (Stat5a$^{-/-}$) mice exhibit defective prolactin-related functions, with impaired lobuloalveolar outgrowth of mammary epithelium during pregnancy, resulting in defective lactation (Liu et al., 1997), whereas Stat5b$^{-/-}$ mice exhibit defective growth similar to that found in Laron dwarfism (Udy et al., 1997). These different phenotypes underscore the distinct roles of Stat5a and Stat5b. Recently, bone marrow–derived macrophages from Stat5a$^{-/-}$ mice were analyzed and shown to exhibit defective GM-CSF–induced proliferation and gene expression (Feldman et al., 1997).

We have now analyzed the immunological properties in Stat5a$^{-/-}$ mice. Given the role of IL-7 in T and B cell development (Peschon et al., 1994; von Freeden-Jeffry et al., 1995) and of IL-2 in the proliferation of peripheral T cells (Smith, 1988), we were particularly interested in analyzing whether lymphoid development is normal in Stat5a$^{-/-}$ mice and whether T cells exhibit normal proliferation. We found that although thymic development was relatively normal, splenocytes were diminished in number and their proliferation in response to low concentrations of IL-2 was diminished due to a defect in IL-2–induced expression of IL-2 receptor α chain (IL-2Rα). However, concentrations of IL-2 sufficient to titrate intermediate affinity IL-2 receptors (which contain IL-2Rβ and the common cytokine receptor γ chain [γ_c] but not IL-2Rα) could induce maximal proliferation, indicating that Stat5a$^{-/-}$ lymphocytes were not defective in their intrinsic ability to proliferate. Given that a number of STATs have been implicated in proliferation, these data reveal an important instance in which the role of a STAT protein in proliferation is indirect. Moreover, they provide evidence for the physiological importance of IL-2–induced up-regulation of IL-2Rα expression.

Results

Because Stat5a is activated in response to IL-7 (Lin et al., 1995) and IL-7 is known to be vital for T cell and B

‖To whom correspondence should be addressed (e-mail: wjl@helix.nih.gov).

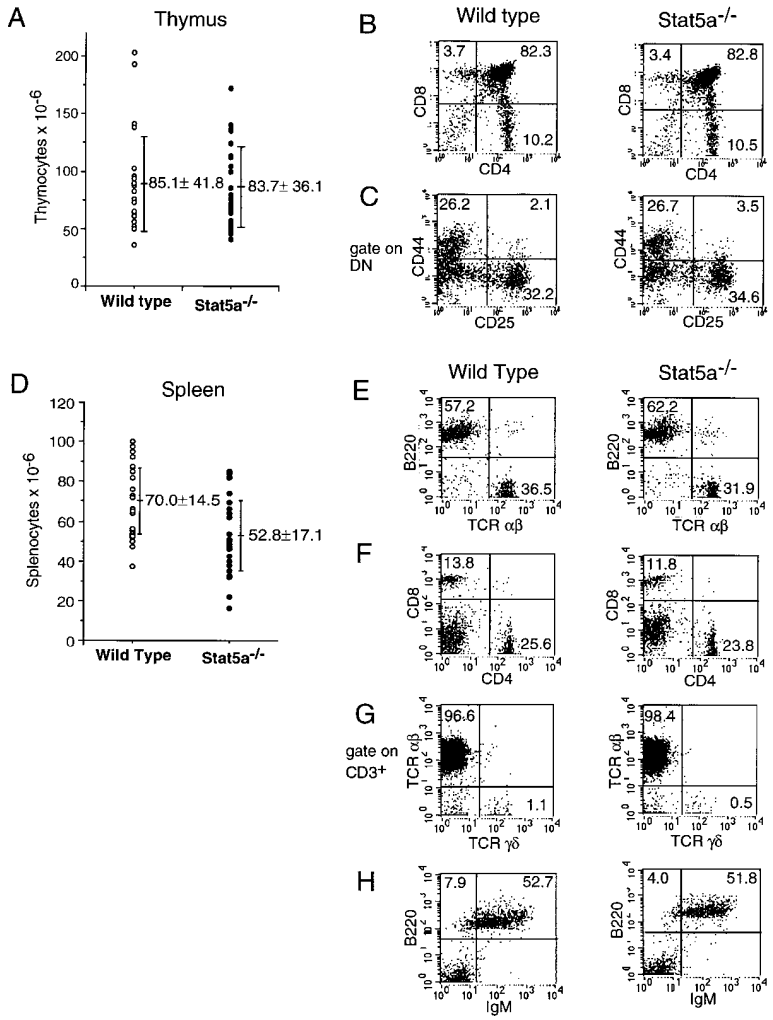

Figure 1. T Cell and B Cell Development in Stat5a$^{-/-}$ Mice

(A) Cellularity of thymus of 5- to 8-week-old wild-type and Stat5a$^{-/-}$ mice.

(B and C) Flow cytometric analysis of thymocytes from 6-week-old mice. (B) Expression of CD4 versus CD8 on total thymocytes using anti-CD8-Cy-Chrome and anti-CD4-FITC. (C) Expression of CD25 versus CD44 on double-negative thymocytes. Single- and double-positive cells were gated out using anti-CD4-FITC and anti-CD8-FITC. Double-negative cells were analyzed using anti-CD44-Cy-Chrome and anti-CD25-PE.

(D) Stat5a$^{-/-}$ mice tended to express slightly diminished numbers of splenocytes. The mice analyzed were 5–8 weeks old.

(E–H) Flow cytometric analysis of splenocytes from 6-week-old mice. Cells were stained with anti-B220 Cy-Chrome versus anti-TCRb (H57)-FITC (E), anti-CD8 Cy-Chrome versus anti-CD4 FITC (F), anti-TCR$\gamma\delta$ FITC versus anti-TCRb Cy-Chrome (gated on CD3$^+$ cells) (G), or anti-B220 Cy-Chrome versus anti-IgM FITC (H).

cell development (Peschon et al., 1994; von Freeden-Jeffry et al., 1995), we first evaluated whether lymphoid development was normal in Stat5a$^{-/-}$ mice. Analysis of the thymus revealed essentially normal cellularity in these mice (Figure 1A; 85.1 $\pm$ 41.8 $\times$ 10^6 for wild-type mice vs. 83.7 $\pm$ 36.1 $\times$ 10^6 for Stat5a$^{-/-}$ mice). In addition, flow cytometric analysis revealed essentially indistinguishable CD4/CD8 staining (Figure 1B), normal IL-2Rα (CD25)/CD44 staining within double-negative CD4$^-$ CD8$^-$ thymocytes (Figure 1C), and normal expression of the CD69 activation antigen on both CD4$^+$CD8$^-$ and CD4$^-$CD8$^+$ single-positive thymocytes (data not shown). In contrast to the normal thymic cellularity in Stat5a$^{-/-}$ mice, the number of splenocytes was modestly decreased (Figure 1D; 70.0 $\pm$ 14.5 $\times$ 10^6 cells/spleen for wild-type mice vs. 52.8 $\pm$ 17.1 $\times$ 10^6 cells/spleen for Stat5a$^{-/-}$ mice, $P < 0.001$). Flow cytometric analysis of splenocyte populations revealed relatively similar T:B cell ratios (based on T cell receptor [TCR] $\alpha\beta$ and B220 expression) in wild-type and Stat5a$^{-/-}$ mice (Figure 1E). Among the T cells, the CD4:CD8 ratios were similar (Figure 1F) and the expression of activation markers (CD69, CD62L, and CD25 [IL-2Rα]) on T cells were normal (data not shown), in contrast to the activated phenotype found in IL-2$^{-/-}$ (Sadlack et al., 1995) and IL-2R$\alpha^{-/-}$ mice (Willerford et al., 1995). T cells bearing $\gamma\delta$ TCR appeared to be diminished in Stat5a$^{-/-}$ mice (Figure 1G). Based on B220 versus immunoglobulin M (IgM) staining, B cells in the spleen (Figure 1H) and bone marrow (data not shown) exhibited relatively normal maturation. Cells of the monocyte/macrophage (Mac-1$^+$ cells) and granulocytic (Gr-1$^+$ cells) lineages also were present in normal numbers (data not shown). The presence of normal T cell populations in thymus and of normal B cell populations in both bone marrow and spleen underscored the ability of IL-7 to act as a thymocyte maturation factor as well as a pre–B cell growth factor in the absence of Stat5a.

We next analyzed STAT activation in Stat5a$^{-/-}$ cells. As shown in Figure 2A, IL-7–induced STAT DNA binding was reduced in Stat5a$^{-/-}$ thymocytes. Analogously, IL-2 could activate STAT DNA binding activity in Stat5a$^{-/-}$ splenocytes, and again less binding activity was seen than in wild-type mice (Figure 2B). Supershifting with an antiserum specific for Stat5 proteins confirmed that the complex induced in Stat5a$^{-/-}$ cells

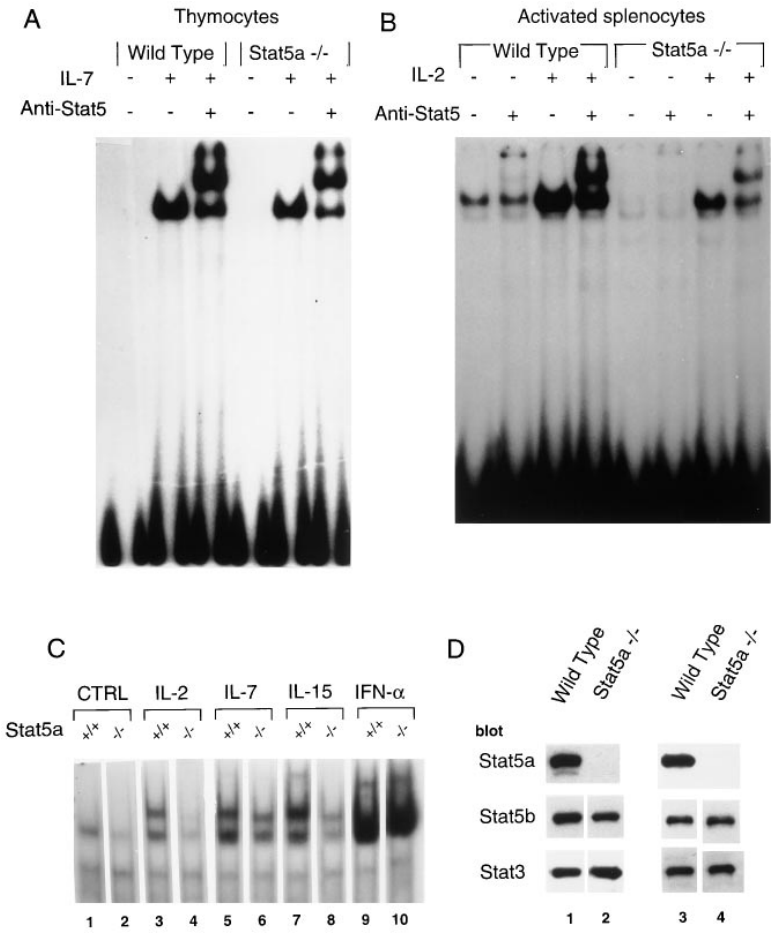

Figure 2. Impaired STAT Activation in Stat5a$^{-/-}$ Thymocytes and Splenocytes

(A) Electromobility shift assays (EMSAs) using extracts from thymocytes that were stimulated with 5 ng/ml (330 pM) of murine recombinant IL-7 for 15 min. Whole-cell extracts were prepared and EMSAs were performed as described in Experimental Procedures.

(B) EMSAs using extracts from activated splenocytes. Anti-CD3–stimulated splenocytes were rested in RPMI 1640 media containing 10% FCS and 2 mM glutamine for 10 hr and stimulated with 2 nM human recombinant IL-2 for 15 min. Whole-cell extracts were prepared and EMSAs were performed.

(C) Splenocytes were activated with phytohemagglutinin and then either not stimulated (control, CTRL) or stimulated with IL-2, IL-7, IL-15, or interferon-α (IFN-α). Extracts were then prepared and EMSAs were performed.

(D) Western blotting of whole-cell extracts from thymocytes treated with IL-7 (lanes 1 and 2) and anti-CD3–preactivated splenocytes treated with IL-2 (lanes 3 and 4) with antisera for Stat5a, Stat5b, and Stat3.

contained Stat5b (Figures 2A and 2B). These results indicated that Stat5b DNA binding activity is not dependent on the presence of Stat5a. Interestingly, like IL-2, IL-15 also induced much less STAT binding activity in the Stat5a$^{-/-}$ splenocytes than in wild-type splenocytes, whereas IL-7–induced STAT binding activity was less impaired (Figure 2C). Since the IL-2 and IL-15 receptors both share the IL-2 receptor β chain (Bamford et al., 1994; Giri et al., 1994), which mediates Stat5 docking and activation (Fujii et al., 1995; Gaffen et al., 1995; Lin et al., 1995; Friedmann et al., 1996), it is logical that STAT protein activation in response to these cytokines would be similar. IL-7–induced Stat5 activation instead is mediated by the IL-7 receptor α chain, which contains in its cytoplasmic domain a tyrosine-based motif that is similar but slightly different from that found in IL-2Rβ (Lin et al., 1995). It is possible that the differences in the sequences of the IL-2Rβ and IL-7Rα motifs could be responsible for an altered efficiency for recruitment and activation of Stat5b. In contrast to the diminished STAT protein activation in response to IL-2, IL-7, and IL-15, there was normal STAT protein activation in response to interferon-α (Figure 2C), which is known to activate primarily complexes containing Stat1 and Stat2 (Schindler and Darnell, 1995), confirming that the Stat5a$^{-/-}$ splenocytes were capable of a full response to a different stimulus. Stat5a$^{-/-}$ mice express normal levels of Stat3 (Figure 2D). In some Stat5a$^{-/-}$ mice, splenocytes expressed normal levels of Stat5b protein (Figure 2D), whereas in others, Stat5b was diminished (data not shown; see Discussion).

Since Stat5a has been shown to interact with glucocorticoid receptor (Stocklin et al., 1996), we next analyzed the dexamethasone-induced death of thymocytes in Stat5a$^{-/-}$ mice. Dexamethasone is known to induce apoptosis of thymocytes, and IL-7 could prevent the dexamethasone-induced death of CD4^{+}CD8^{-} mature thymocytes in wild-type mice (Figure 3, left). The induction of death by dexamethasone and its prevention by IL-7 were also seen in Stat5a$^{-/-}$ mice at a level similar to that observed in wild-type mice (Figure 3, right). These results indicate that Stat5a is not required for dexamethasone-induced death and that at least some of the signals induced by IL-7 for preventing dexamethasone-induced death are not absolutely dependent on Stat5a.

Given the importance of IL-2 in T cell proliferation and the ability of IL-2 to activate Stat5a potently (Beadling, et al., 1994; Fujii et al., 1995; Gaffen et al., 1995; Gilmour et al., 1995; Hou et al., 1995; Lin et al., 1995; Wakao et al., 1995; Lin et al., 1996), we next examined T cell proliferation in the spleen. Substantial proliferation of fresh splenocytes was observed in response to anti-CD3 (Table 1). In this and other experiments, although the difference was not statistically significant, cells from the

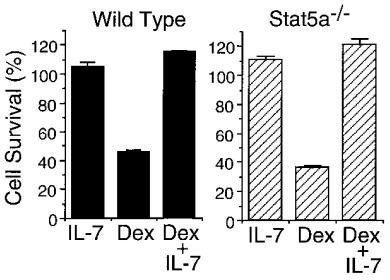

Figure 3. Stat5a$^{-/-}$ Mice Exhibit Normal Dexamethasone-Induced Death of CD4^{+}CD8^{-} Thymocytes and Normal Prevention of This Death by IL-7

Thymocytes (1×10^5) from Stat5a$^{-/-}$ or wild-type mice were cultured with murine recombinant IL-7 (5 ng/ml) and/or dexamethasone (Dex) (2×10^{-8} M). Eighteen hours later, cell viability was determined as described in Experimental Procedures. Shown are means ± SD from three mice.

Table 1. Splenocyte Proliferation

Genotype	RPMI	Anti-CD3
+/+	1299 ± 260	74,516 ± 7888
+/+	635 ± 25	55,258 ± 3797
+/+	756 ± 69	92,967 ± 5792
−/−	219 ± 54	65,564 ± 22,753
−/−	180 ± 64	47,859 ± 19,847
−/−	125 ± 49	25,860 ± 4582

Splenocytes from Stat5a$^{-/-}$ mice proliferated in response to anti-CD3. Shown is the mean [^{3}H]thymidine uptake ± SD of triplicate wells.

Stat5a$^{-/-}$ mice tended to exhibit less proliferation in response to anti-CD3 than did cells from the wild-type mice. Since anti-CD3–induced IL-2 production in Stat5a$^{-/-}$ mice was normal (data not shown), we speculated that a partial defect in proliferation might result from diminished cellular sensitivity to IL-2. Indeed, when Stat5a$^{-/-}$ splenocytes that had been preactivated with anti-CD3 were stimulated with IL-2, proliferation was markedly diminished at low concentrations of IL-2 but was much more similar to that seen in wild-type mice at high concentrations of IL-2 (Table 2). The finding that high concentrations of IL-2 were required for achieving normal levels of proliferation suggested that IL-2Rα expression might be compromised in the Stat5a$^{-/-}$ mice, thereby decreasing the number of high affinity IL-2 receptors. This hypothesis was consistent with a Stat5 binding site in an IL-2 response element in the IL-2Rα gene (Sperisen et al., 1995; John et al., 1996; Lecine et al., 1996). Whereas anti-CD3–induced levels of IL-2Rα protein expression were only modestly diminished in Stat5a$^{-/-}$ CD8^{+} (Figure 4A) and CD4^{+} (Figure 4B) splenic T cells as compared to wild-type cells (compare histograms b and g in each panel), there was a substantial defect in IL-2–induced IL-2Rα expression even though we used a high enough concentration of IL-2 (2 nM) to signal through intermediate affinity receptors (compare histograms e and j in each panel). For example, Stat5a$^{-/-}$ CD8^{+} T cells exhibited approximately 40%–45% of the wild-type level of IL-2Rα after stimulation with anti-CD3 (448.3 vs. 1051.1 mean fluorescent intensity [Figure 4A, histogram g vs. b]). However, when these activated cells were washed and stimulated with IL-2 for an additional 48 hr, IL-2Rα expression was approximately 10%–15% of wild-type (mean fluorescent intensity of 154.8 for Stat5a$^{-/-}$ mice vs. 1295.6 for wild-type mice) (Figure 4A, histogram j vs. e). A similar though less dramatic defect was seen in CD4^{+} T cells (Figure 4B). Expression of another activation marker (CD69), which is not affected by IL-2 stimulation, was equivalently expressed in wild-type and Stat5a-deficient mice under all treatment conditions (Figure 4C). Expression of another inducible gene, IL-2Rβ, was also examined and similar levels of IL-2Rβ were observed in wild-type and Stat5a$^{-/-}$ mice under all conditions (Figure 4D, see legend). The diminished IL-2Rα protein expression corresponded to diminished levels of IL-2Rα mRNA (Figures 5A and 5B). In view of the decreased expression of IL-2Rα, we also examined the expression of other IL-2–induced genes (Figure 5). Although levels of both IL-2Rβ and Bcl-2 mRNA were diminished, their expression was more normal than that seen for IL-2Rα. Expression of IL-2, which is not regulated by IL-2, was not diminished.

Finally, we analyzed the clonal expansion of T cells in Stat5a$^{-/-}$ mice using the bacterial superantigen staphylococcal enterotoxin B (SEB) (Kawabe and Ochi, 1991). In this system, following SEB administration, the

Table 2. Proliferation of Anti-CD3–Stimulated Splenocytes

			IL-2		
Experiment	Genotype	RPMI	10 pM	100 pM	1000 pM
Experiment 1	+/+	118,640 ± 9348	136,327 ± 1830	180,671 ± 9760	311,122 ± 9380
	+/+	116,856 ± 6619	108,786 ± 4790	169,265 ± 6590	255,882 ± 8377
	−/−	36,585 ± 1078	39,200 ± 1312	78,419 ± 4866	218,462 ± 9273
	−/−	22,226 ± 975	30,711 ± 1687	72,722 ± 2733	248,836 ± 6234
	−/−	38,087 ± 5777	31,080 ± 620	70,013 ± 5875	178,744 ± 8609
	−/−	55,113 ± 4868	54,022 ± 1939	93,471 ± 1941	266,789 ± 8281
Experiment 2	+/+	222,223 ± 3471	215,156 ± 5000	230,787 ± 5281	282,810 ± 11,912
	−/−	30,928 ± 3609	36,498 ± 10048	70,080 ± 4273	219,775 ± 16,638
	−/−	48,648 ± 4091	53,896 ± 5013	113,095 ± 2383	358,991 ± 9360
	−/−	67,267 ± 4141	78,171 ± 6004	111,651 ± 3651	286,339 ± 12,485

Impaired proliferation of anti-CD3–stimulated Stat5a$^{-/-}$ splenocytes at low concentration of IL-2. Wild type and Stat5a$^{-/-}$ splenocytes were stimulated in Falcon 3003 plates coated with 10 μg/ml anti-CD3 mAb for 48 hr and washed twice with PBS, and cells (1×10^5/well) were cultured with the indicated concentrations of human recombinant IL-2 for 24 hr with [^{3}H]thymidine added during the final 10 hr.

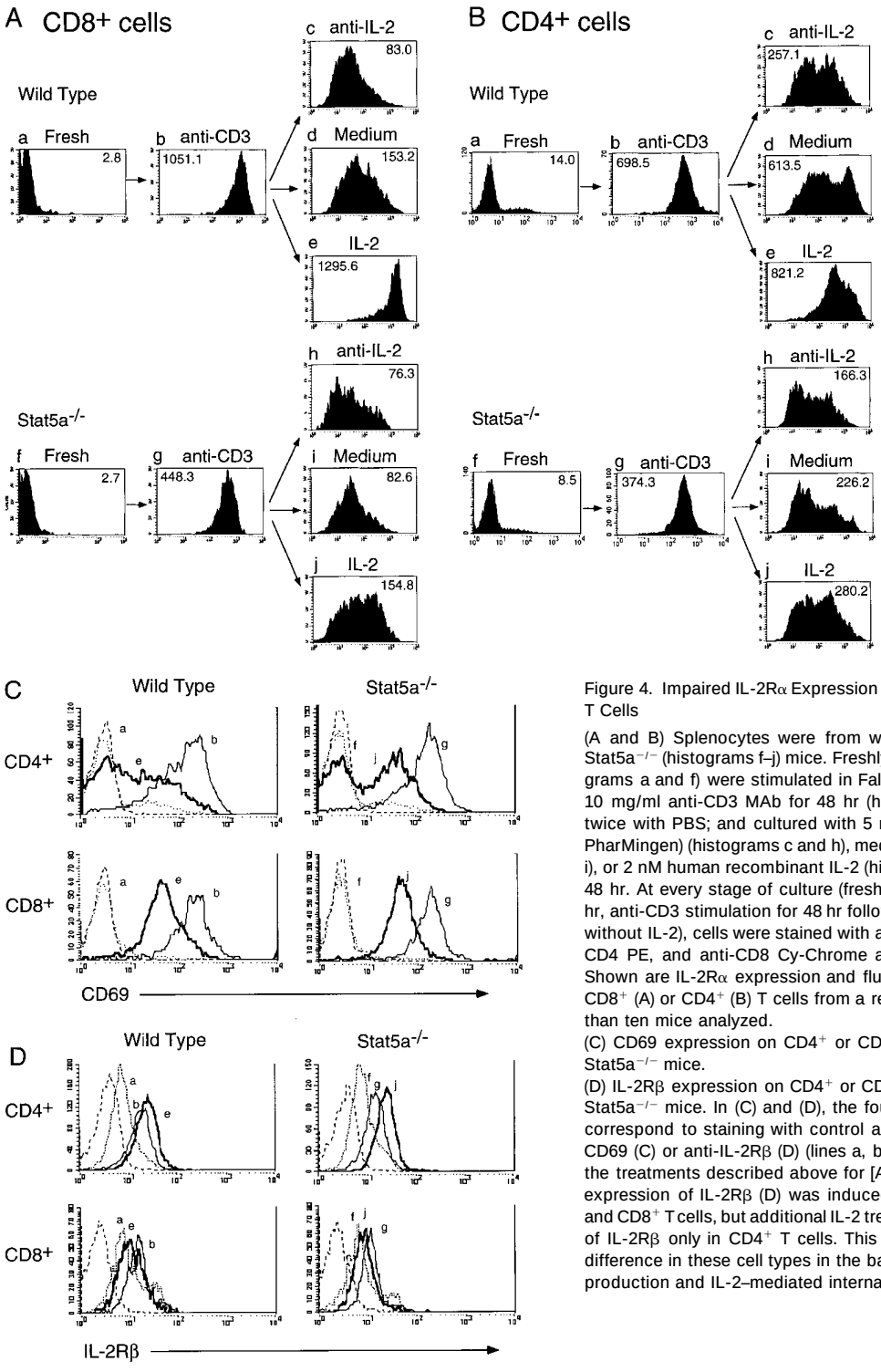

Figure 4. Impaired IL-2Rα Expression on Activated $CD8^+$ and $CD4^+$ T Cells

(A and B) Splenocytes were from wild-type (histograms a–e) or $Stat5a^{-/-}$ (histograms f–j) mice. Freshly isolated splenocytes (histograms a and f) were stimulated in Falcon 3003 plates coated with 10 mg/ml anti-CD3 MAb for 48 hr (histograms b and g); washed twice with PBS; and cultured with 5 mg/ml anti-IL-2 (clone S4B6, PharMingen) (histograms c and h), medium alone (histograms d and i), or 2 nM human recombinant IL-2 (histograms e and j) for another 48 hr. At every stage of culture (fresh, anti-CD3 stimulation for 48 hr, anti-CD3 stimulation for 48 hr followed by another 48 hr with or without IL-2), cells were stained with anti-IL-2Rα (CD25) FITC, anti-CD4 PE, and anti-CD8 Cy-Chrome and analyzed on a FACSort. Shown are IL-2Rα expression and fluorescent intensity (mean) on $CD8^+$ (A) or $CD4^+$ (B) T cells from a representative mouse of more than ten mice analyzed.

(C) CD69 expression on $CD4^+$ or $CD8^+$ T cells from wild-type or $Stat5a^{-/-}$ mice.

(D) IL-2Rβ expression on $CD4^+$ or $CD8^+$ T cells from wild-type or $Stat5a^{-/-}$ mice. In (C) and (D), the four superimposed histograms correspond to staining with control antibody (broken line) or anti-CD69 (C) or anti-IL-2Rβ (D) (lines a, b, e, f, g, and j correspond to the treatments described above for [A] and [B]). Note that surface expression of IL-2Rβ (D) was induced by anti-CD3 in both $CD4^+$ and $CD8^+$ T cells, but additional IL-2 treatment increased expression of IL-2Rβ only in $CD4^+$ T cells. This presumably resulted from a difference in these cell types in the balance between IL-2–induced production and IL-2–mediated internalization of IL-2Rβ.

T cells bearing Vβ8 T cell receptors are specifically activated and expanded in vivo and subsequently deleted by apoptosis. As shown in Figure 6A, there was little, if any, SEB-induced expansion of $V\beta8^+$ $CD8^+$ T cells in $Stat5a^{-/-}$ mice ($P < 0.01$), whereas SEB-induced expansion of $V\beta8^+$ $CD4^+$ T cells occurred albeit less potently than that seen in wild-type mice ($p = 0.052$, Figure 6B). The expected compensatory changes were seen in $V\beta6^+$ T cells in both wild-type and $Stat5a^{-/-}$ mice (Figures 6C and 6D). Because mice lacking IL-2 (Kneitz et al., 1995) or IL-2Rα (Willerford et al., 1995) are defective in SEB-induced deletion of $V\beta8^+$ T cells, we analyzed SEB-induced deletion in $Stat5a^{-/-}$ mice. Of note, no detectable defect was found in SEB-induced deletion in

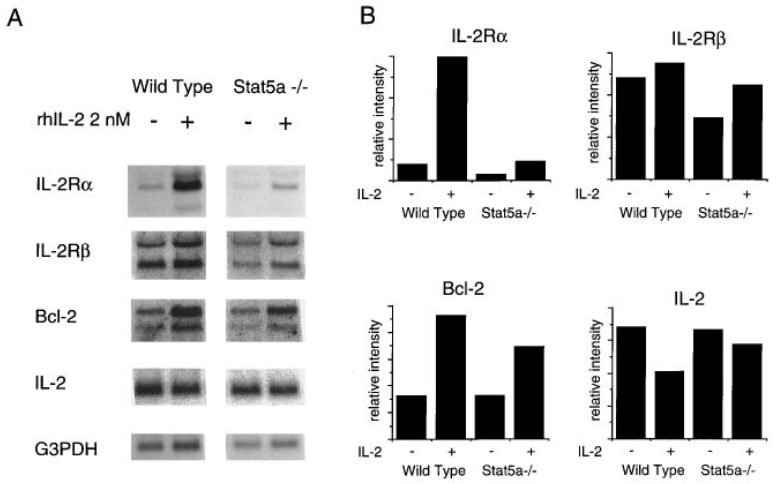

Figure 5. Impaired IL-2Rα mRNA Expression in Activated Splenocytes

(A) Anti-CD3–stimulated splenocytes were cultured with or without 2 nM human recombinant IL-2 for 10 hr. Cells were washed with PBS, and RNA was extracted using Trizol reagent. Northern blots were hybridized with the indicated genes.

(B) The intensities of bands were quantified by densitometry and their intensities were normalized relative to the amount of G3PDH hybridization. Shown are representative data from four mice.

Stat5a$^{-/-}$ mice at day 8 (Figures 6A and 6B). Consistent with this finding, SEB could increase apoptosis of Vβ8^{+} T cells as evaluated by staining with Annexin V (Figure 7A) and induce Fas expression on Vβ8^{+} T cells (Figure 7B) to a similar level in Stat5a$^{-/-}$ and wild-type mice. This Fas induction was functional, since Fas-mediated death was enhanced by SEB treatment in both Vβ8^{+} CD4^{+} T cells (Figures 7C and 7E) and Vβ8^{+} CD8^{+} T cells (Figures 7D and 7F) with a similar efficiency in Stat5a$^{-/-}$ mice and wild-type littermates. This was seen with either agonistic anti-Fas monoclonal antibody (MAb) (clone Jo2, Figures 7C and 7D) or soluble Fas ligand (FasL) (Figures 7E and 7F). These results indicate that Stat5a is required for normal levels of SEB-induced expansion (presumably by allowing IL-2–induced IL-2Rα expression). In contrast, Stat5a-mediated signaling is not required for maintaining normal levels of SEB-induced deletion.

Discussion

There are three classes of IL-2 receptors, binding IL-2 with low ($K_d = 10^{-8}$ M), intermediate ($K_d = 10^{-9}$ M), and high ($K_d = 10^{-11}$ M) affinities. Low-affinity IL-2 receptors contain only IL-2Rα; intermediate-affinity receptors contain IL-2Rβ and γ_c; and high-affinity receptors contain all three chains. Only the intermediate and high-affinity receptors can transduce signals in response to IL-2. Consistent with this observation, the heterodimerization of IL-2Rβ and γ_c is both necessary and sufficient for IL-2 signaling (Nakamura et al., 1994; Nelson et al., 1994). Although signaling can occur in the absence of IL-2Rα, IL-2Rα is vital for normal immune function, as demonstrated by the autoimmunity, inflammatory bowel disease, and premature death exhibited by IL-2Rα$^{-/-}$ mice (Willerford et al., 1995) and by the severe combined immunodeficiency in a patient with IL-2Rα deficiency (Sharfe et al., 1997). Presumably, IL-2Rα is required to convert intermediate-affinity receptors into high-affinity receptors, thus conferring a 100-fold increase in binding affinity for IL-2, and allowing efficient cellular responsiveness to the low concentrations of IL-2 that are physiologically produced in vivo.

IL-2Rα expression is induced by a variety of stimuli, including antigens/mitogens and certain cytokines, such as IL-2 (Leonard et al., 1984, 1994; Depper et al., 1985; reviewed by Smith, 1988). Analysis of the 5′ regulatory region of the IL-2Rα gene has revealed the presence of at least three important regulatory regions. Whereas positive regulatory regions I and II are essential for mitogen-induced IL-2Rα expression (John et al., 1995), they are insufficient for IL-2–induced regulation of the IL-2Rα gene. Instead, a third region, positive regulatory region III, which binds both Stat5 and Elf-1, forms a potent IL-2 response element in both the murine (Sperisen et al., 1995) and human (John et al., 1996; Lecine et al., 1996) IL-2Rα genes. Indeed, mutations of tyrosines in IL-2Rβ that are required for Stat5 docking and activation are required for IL-2Rα induction (Friedmann et al., 1996; Ascherman et al., 1997). Our data now establish that Stat5a is vital for normal IL-2–induced IL-2Rα expression but not for anti-CD3–induced IL-2Rα expression. The finding that anti-CD3–induced IL-2Rα expression was diminished in Stat5a$^{-/-}$ mice may be explained by the fact that anti-CD3 stimulation also induces IL-2 production; thus, anti-CD3 has both "direct" TCR-mediated effects and "indirect" IL-2–induced effects on IL-2Rα induction in normal mice, but in the Stat5a$^{-/-}$ mice, these latter signals are defective. Moreover, our data indicate that proliferation at physiological levels of IL-2 is dependent on IL-2–induced IL-2Rα expression and suggest that antigen-mediated induction of IL-2Rα, without IL-2–induced maintenance of IL-2Rα levels, is insufficient for normal proliferation or expansion of T cells in vivo.

Considerable discussion has centered on the role of STATs in proliferation (Leonard, 1996). Both Stat4 (Kaplan et al., 1996b; Thierfelder et al., 1996) and Stat6 (Kaplan et al., 1996a; Shimoda et al., 1996; Takeda et al., 1996) knockout mice exhibit diminished proliferation in response to IL-12 and IL-4, respectively, although the mechanism(s) for these effects have remained unclear. In the case of Stat6$^{-/-}$ mice, IL-4Rα expression is diminished, making it unclear whether the lack of Stat6 had a direct or indirect effect on proliferation. Various studies have either suggested no role (Quelle et al., 1996) or some role (Goldsmith et al., 1995; Mui et al., 1995a; Friedmann et al., 1996) for Stat5 in proliferation. Our

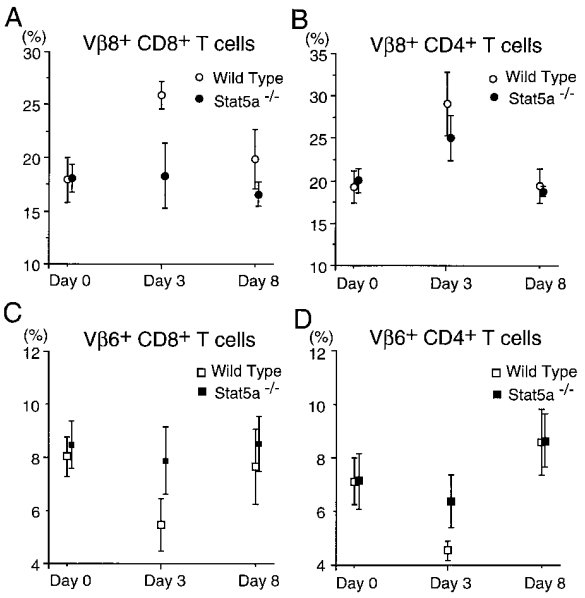

Figure 6. Defective SEB-Induced Expansion of Vβ8+ T Cells in Stat5a$^{-/-}$ Mice

Wild-type or Stat5a$^{-/-}$ mice were injected with SEB as described in Experimental Procedures. The percentage Vβ8 (A and B) and Vβ6 (C and D) were then determined for CD8 (A and C) and CD4 (B and D) populations of T cells.

data clarify that at least for IL-2 signaling, the defect in proliferation in Stat5a$^{-/-}$ lymphocytes is indirect and is based on diminished IL-2Rα expression, since maximal proliferation in vitro could be achieved at levels of IL-2 sufficient to titrate IL-2Rβ/γ_c intermediate affinity receptors. The defect in SEB-induced clonal expansion of Vβ8$^+$ T cells in Stat5a$^{-/-}$ mice, particularly among the CD8$^+$ T cells, indicates that Stat5a is vital for clonal expansion in vivo, at least for the CD8$^+$ T cells (the cells in which we observed a greater defect in IL-2–induced IL-2Rα expression [Figure 4]).

Not only is IL-2Rα expression important for maximal T cell proliferation; it also appears to play a role in sensitizing T cells to activation-induced cell death, since this function is defective in IL-2Rα–deficient mice (Willerford et al., 1995). Presumably, because of this defect of activation-induced cell death, IL-2Rα–deficient mice exhibit lymphadenopathy and autoimmune disease. It is therefore noteworthy that the Stat5a$^{-/-}$ mice (at least until 4 months of age, the oldest mice we have examined) develop neither lymphadenopathy nor an activated phenotype of T cells (data not shown), as is typically found in the IL-2Rα–deficient mice (Willerford et al., 1995). The finding that the Stat5a$^{-/-}$ mice are more normal than IL-2Rα$^{-/-}$ mice might be explained by their retention of relatively normal anti-CD3–induced IL-2Rα expression.

Stat5b expression was diminished in some Stat5a$^{-/-}$ mice. This could suggest that Stat5b expression can be influenced by Stat5a or by a Stat5a-regulated gene product, and it was possible that diminished Stat5b expression could contribute to the defects observed. However, even Stat5a$^{-/-}$ mice with normal levels of Stat5b exhibited the same defects, indicating that defective

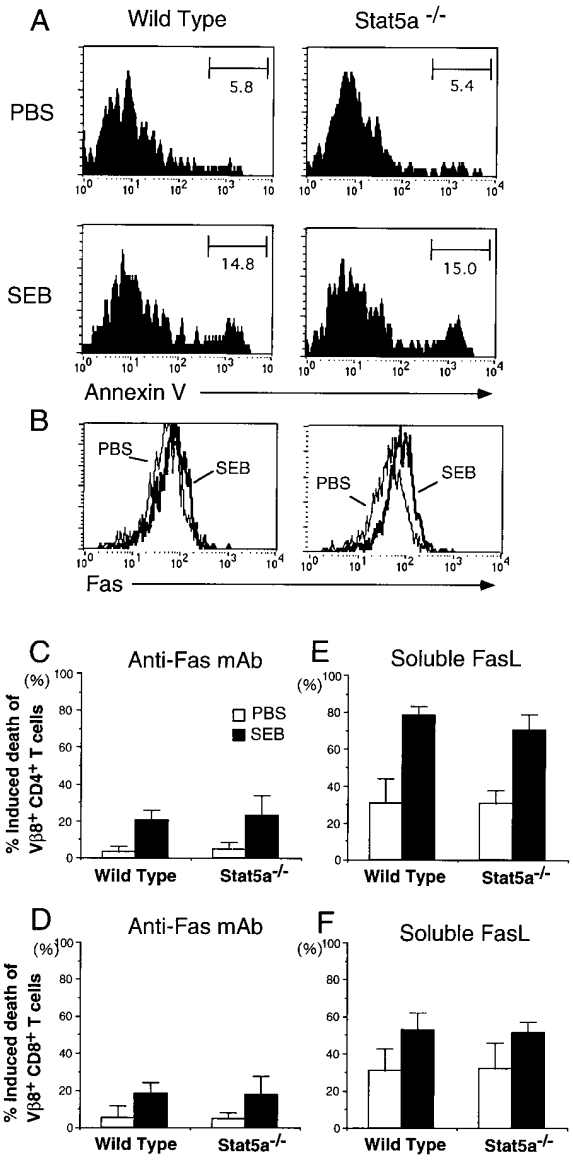

Figure 7. Stat5a-Deficient Mice Exhibit Normal Fas-Mediated Death

(A) Similar levels of annexin V staining in wild-type and Stat5a-deficient Vβ8$^+$CD4$^+$ T cells in mice treated with PBS (top) or SEB (bottom) for 5 days.

(B) Basal (PBS) and SEB-induced levels of Fas expression was similar in wild-type and Stat5a$^{-/-}$ mice. Shown is anti-Fas staining on Vβ8$^+$CD4$^+$ T cells in mice treated with PBS or SEB for 5 days.

(C–F) Mice were pretreated for 72 hr with PBS (open bars) or SEB (filled bars) before cells were harvested as described in Experimental Procedures. (C and D) Shown are means ± SD of the percentage induced cell death in Vβ8$^+$ CD4$^+$ (C) and Vβ8$^+$ CD8$^+$ (D) T cells treated with anti-Fas MAb (clone Jo2). (E and F) Shown are means ± SD of percentage induced cell death in Vβ8$^+$ CD4$^+$ (E) and Vβ8$^+$ (F) T cells treated with soluble FasL.

Stat5a expression itself was responsible for diminished induction of IL-2Rα in response to IL-2.

Given that Stat5a is activated by IL-7 (Lin et al., 1995), it was striking that no obvious developmental abnormalities were found in the immune systems of Stat5a$^{-/-}$ mice, even though IL-7$^{-/-}$ and IL-7Rα$^{-/-}$ mice exhibit

markedly compromised T cell and B cell development (Peschon et al., 1994; von Freeden-Jeffry et al., 1995). In addition, although IL-7 is known to play a role in IL-2Rα expression in double-negative thymocytes (Zuniga-Pflucker et al., 1996), Stat5a$^{-/-}$ mice exhibit normal IL-2Rα expression in these cells. Thus, Stat5a is not required for these actions of IL-7. We have also shown that the absence of Stat5a had little effect on the ability of IL-7 to prevent dexamethasone-induced apoptosis. Given that Stat5a and the glucocorticoid receptor can physically interact (Stocklin et al., 1996), it might have been expected that dexamethasone-induced apoptosis and its prevention by IL-7 would be impaired in Stat5a$^{-/-}$ mice. Thus, either the Stat5a-glucocorticoid receptor interaction is not important for this apoptosis or there are redundant pathways. Since IL-4 can also exhibit a protective effect similar to that of IL-7 in preventing dexamethasone-induced apoptosis (Migliorati et al., 1993), even though IL-4 activates Stat6 instead of Stat5 proteins, it is clear that protective effects against dexamethasone-induced apoptosis are not strictly dependent on a particular STAT protein.

Therefore, from the analysis of Stat5a-deficient mice, we can draw several conclusions. First, we provide the first example in which the mechanism of defective proliferation in a STAT-deficient system is clearly an indirect effect, based on the regulation of expression of a component of a cytokine receptor chain (IL-2Rα). Defective Stat5a expression did not cause a defect in the intrinsic proliferative potential of peripheral T cells; instead, it caused a defect IL-2–induced IL-2Rα expression, which in turn caused defective T cell proliferation in vitro at low levels of IL-2. Second, the finding that superantigen-induced T cell expansion in vivo was also defective demonstrates the importance of IL-2–induced IL-2Rα expression in vivo. Third, given the severe defect in thymic and B cell development in mice deficient in IL-7 or IL-7Rα (and in mice deficient in γ_c [Cao et al., 1995; DiSanto et al., 1995; Ohbo et al., 1996] or Jak3 [Nosaka et al., 1995; Park et al., 1995; Thomis et al., 1995]), our data indicate that Stat5a is not an absolutely essential downstream signaling molecule for IL-7 in lymphoid development, even though IL-7 potently activates Stat5a. Fourth, our data provide in vivo evidence that there are two mechanisms by which the IL-2Rα gene can be activated (one by anti-CD3, largely independent of Stat5a, and one by IL-2, largely dependent on Stat5a). Considering the data reported by Willerford et al. (1995) on the phenotype of IL-2Rα–deficient mice, we can now conclude that there are important physiological roles for both mechanisms of activation of the IL-2Rα gene. Fifth, the finding that both IL-2 and Stat5a-deficient mice exhibit defective expansion of cells but that only IL-2–deficient mice exhibit defective deletion suggests that Stat5a plays a vital role in expansion but not in FasL-mediated deletion and that Stat5a-independent pathways are responsible for the differences in deletion. Finally, these mice provide insight into the immunological phenotype that might be seen in humans with Stat5a deficiency, an important consideration in the treatment of patients with primary immunodeficiencies associated with normal numbers of T and B cells.

Experimental Procedures

Mice

All animals used for these experiments were F2 or F3 animals derived from Stat5a heterozygous crosses of mixed background (129 SvEv × NIH Black Swiss or 129 SvEv × BALB/c) animals. All experiments were carried out under protocols approved by National Institutes of Health Animal Use and Care Committees and followed the National Institutes of Health guidelines "Using Animals in Intramural Research."

Flow Cytometric Analyses

Cells from the thymus and spleen were stained and analyzed on a FACSort (Becton Dickinson, San Jose, CA) using CELLQuest software (Cao et al., 1995). For direct staining, the following conjugated antibodies were purchased from PharMingen (San Diego, CA): anti-TCRαβ fluorescein isothiocyanate (FITC) and Cy-chrome (H57–597 to TCRβ); anti-TCRγδ FITC (GL3), anti-CD3ε phycoerythrin (PE) (145–2C11), anti-CD4 FITC, PE, and Cy-Chrome (H129.19); anti-CD8 FITC, PE, and Cy-Chrome (53–6.7); anti-IL-2Rα (CD25) FITC (7D4), anti-IL-2Rα PE (3C7), anti-CD44 Cy-Chrome (IM7), anti-CD45R (B220) Cy-Chrome (RA3–6B2), anti-CD69 PE (H1.2F3), anti-IgM FITC (R6–60.2), anti-IL-2Rβ FITC (TM-β1), anti-TCR Vβ6-FITC, and PE (RR4–7); and anti-TCR Vβ8.1,2 FITC and PE (MR5–2).

Annexin V Staining

After cells were stained with anti-CD4 Cy-Chrome and anti-Vβ8 PE and washed twice with phosphate-buffered saline (PBS) containing 1% bovine serum albumin (BSA), cells were stained with annexin V FITC (R & D Systems) according to the manufacturer's instructions. Cells were analyzed on a FACSort.

Dexamethasone-Induced Cell Death Assay of Thymocytes

An in vitro cell death assay of thymocytes was performed by modifying the method of Negishi et al. (1995). In brief, thymocytes (1×10^5) from Stat5a$^{-/-}$ or wild-type mice were cultured in RPMI 1640 media supplemented with 10% fetal bovine serum (FBS), 2 mM glutamine, and antibiotics. Where indicated, murine recombinant IL-7 (R & D Systems) was added at 5 ng/ml (330 pM), and dexamethasone was used at 2×10^{-8} M. Eighteen hours later, cells were harvested and stained with anti-CD4 FITC and anti-CD8 PE, and cell viability was determined using propidium iodide (PI, 5 μg/ml, Sigma) and a flow cytometer without gating. Cells were analyzed in triplicate. The percentage survival of CD4$^+$CD8$^-$ (CD4 single-positive) cells was calculated as (number of viable CD4 single-positive cells cultured with each additive/number of viable CD4 single-positive cells cultured in medium alone) × 100.

SEB-Induced Expansion and Deletion of Vβ8$^+$ T Cells In Vivo

Mice 3–4 months old were injected intraperitoneally with PBS or 50 μg of staphylococcal enterotoxin B (SEB, Sigma) and then sacrificed on days 3 and 8. Splenocytes were stained with anti-CD4 PE and anti-CD8 Cy-Chrome plus either anti-Vβ8.1,2 FITC or anti-Vβ6 FITC and then analyzed on a FACSort.

Fas-Mediated Cell Death of Splenic T Cells

Stat5a$^{-/-}$ or littermate control mice were injected with PBS or 50 μg of SEB. Mice were sacrificed 72 hr later and sensitivity to Fas-mediated cell death was analyzed in two ways. First, splenocytes were cultured for 6 hr with 5 μg/ml agonistic anti-Fas MAb (clone Jo2, no sodium azide, low endotoxin; PharMingen; Ogasawara et al., 1993) or purified hamster IgG (PharMingen) as a control. Second, splenocytes were cultured for 6 hr with soluble FasL (100 ng/ml) with 1 μg/ml of an enhancer of FasL-mediated killing (Alexis, San Diego, CA) according to the manufacturer's instructions. Cells were then harvested, stained with anti-Vβ8 FITC, and either anti-CD4 CyChrome or anti-CD8 CyChrome. Cell viability was determined using PI and a flow cytometer, without gating. The percentage Fas-induced cell death of CD4$^+$ Vβ8$^+$ T cells was calculated as (1 − [number of CD4$^+$ Vβ8$^+$ PI$^-$ cells in the experimental culture]/[number of CD4$^+$ Vβ8$^+$ PI$^-$ cells in the control culture]) × 100. Analogously,

the percentage Fas-induced cell death of $CD8^+$ $V\beta8^+$ T cells was calculated.

Preactivation of Splenocytes

Splenocytes (1×10^6/ml) were stimulated in Falcon 3003 plates coated with 10 μg/ml anti-CD3ε MAb (145–2C11, PharMingen) or phytohemagglutinin in RPMI 1640 medium containing 10% FBS, 2 mM glutamine, and antibiotics for 48 hr.

IL-2 Production and Proliferation Assays

For IL-2 production, splenocytes were cultured for 48 hr in Falcon 3003 plates coated with 10 μg/ml anti-CD3ε MAb (145–2C11), and IL-2 was measured by bioassay as described previously (Cao et al., 1995). For proliferation of fresh splenocytes, 96-well plates were coated with 10 μg/ml anti-CD3ε MAb. Splenocytes (2×10^5/well) were cultured for 48 hr, with 1 μCi of [^{3}H]thymidine added for the final 10 hr. For proliferation of anti-CD3–stimulated splenocytes, cells (1×10^5) were cultured with various concentration of IL-2 (Cetus) for 24 hr with a [^{3}H]thymidine pulse for the last 10 hr.

Northern Blotting

Anti-CD3–stimulated splenocytes were cultured with or without 2 nM human recombinant IL-2 for 10 hr. Cells were washed with PBS, and RNA was extracted using Trizol reagent (Gibco BRL). Northern blotting was performed using 18 μg of total RNA by standard methods, as previously described (Ascherman et al., 1997). Blots were hybridized with the following probes: a 1.2 kb fragment of murine IL-2Rα cDNA (Miller et al., 1985), a 1.7 kb fragment of murine IL-2Rβ cDNA (a gift from T. Malek), a 2.2 kb fragment of murine Bcl-2 cDNA (a gift from J. F. Mushinski), a 0.5 kb polymerase chain reaction (PCR)–generated fragment of the murine IL-2 cDNA, and a 1.0 kb PCR fragment of the murine glyceraldehyde-3-phosphate dehydrogenase (G3PDH) cDNA (Ascherman et al., 1997). Probes were labeled by random priming using the Prime It II Kit (Stratagene).

Electrophoretic Mobility Shift Assays

A probe derived from the ovine β-casein promoter was labeled with [^{32}P]dGTP and Klenow fragment of DNA polymerase, as previously described (Lin et al., 1996). Cells were washed twice with PBS, resuspended in lysis buffer (20 mM HEPES [pH 7.8], 450 mM NaCl, 0.4 mM EDTA, 0.5 mM dithiothreitol, 25% glycerol, 0.5 mM AEBSF (4-(2-aminoethyl)-benzenesulfonyl fluoride hydrochloride), and protease inhibitors), and subjected to three freeze–thaw cycles. Whole-cell extracts were prepared by spinning at 18,000 × g at 4°C for 15 min. Samples (12 μg) of extract in 10 μl of lysis buffer was combined with 11 μl of 2× binding buffer (100 mM KCl, 20 mM Tris-HCl, 20 mM HEPES, 1 mM dithiothreitol, 1 mM EDTA, 20% glycerol [pH 7.8]) containing 2 μg of poly dI-dC and incubated for 20 min on ice, and then 3×10^4 cpm of the probe was added and incubation continued on ice for an additional 20 min. Supershifting was performed by preincubating the whole-cell extract with 3 μl of a pan-Stat5 antiserum that recognizes both Stat5a and Stat5b (Lin et al., 1995). Electrophoresis was carried out at room temperature using 5% or 6% polyacrylamide gels.

Western Blotting

Samples (15 μg) of whole cell extracts were separated on 8% SDS polyacrylamide gels and transferred to Immobilon-P membranes (Millipore). Blots were blocked with PBS containing 0.15% Tween 20 and 3% BSA for 1 hr at room temperature and incubated with anti-Stat5a (Liu et al., 1996), anti-Stat5b (Liu et al., 1996), or anti-Stat3 (Transduction Laboratories) for 1 hr at room temperature. After three washes of 10 min each with PBS containing 0.15% Tween 20, blots were incubated with anti-mouse or anti-rabbit antibodies conjugated with horseradish peroxidase (Amersham) in PBS containing 0.15% Tween 20 and 3% BSA for 1 hr at room temperature; washed with PBS containing 0.15% Tween 20; and developed with an enhanced chemiluminescent substrate (Pierce).

Acknowledgments

We thank M. Noguchi and D. P. Ascherman for providing probes, G. Feldman for valuable discussions, J.-X. Lin for providing the pan-Stat5 antibody, T. R. Malek for the murine IL-2Rβ cDNA, J. F. Mushinski for the murine bcl-2 cDNA, J. Hu-Li for measuring IL-2 levels, and S. John for critical comments. H. N. was supported in part by Japan Society for the Promotion of Science and The Naito Foundation.

Received March 14, 1997; revised August 13, 1997.

References

Ascherman, D.P., Migone, T.-S., Friedmann, M.C., and Leonard, W.J. (1997). IL-2-mediated induction of the IL-2 receptor α chain gene: critical role of two functionally redundant tyrosine residues in the IL-2 receptor β chain cytoplasmic domain and suggestion that these residues mediate more than Stat5 activation. J. Biol. Chem. *272*, 8704–8709.

Azam, M., Erdjument-Bromage, H., Kreider, B.L., Xia, M., Quelle, F., Basu, R., Saris, C., Tempst, P., Ihle, J.N., and Schindler, C. (1995). Interleukin-3 signals through multiple isoforms of Stat5. EMBO J. *14*, 1402–1411.

Bamford, R.N., Grant, A.J., Burton, J.D., Peters, C., Kurys, G., Goldman, C.K., Brennan, J., Roessler, E., and Waldmann, T.A. (1994). The interleukin (IL)-2 receptor beta chain is shared by IL-2 and a cytokine, provisionally designated IL-T, that stimulates T cell proliferation and the induction of lymphokine activated killer cells. Proc. Natl. Acad. Sci. USA *92*, 4940–4944.

Beadling, C., Guschin, D., Witthuhn, B.A., Ziemiecki, A., Ihle, J.N., Kerr, I.M., and Cantrell, D.A. (1994). Activation of JAK kinases and STAT proteins by interleukin-2 and interferon α, but not the T cell antigen receptor, in human T lymphocytes. EMBO J. *13*, 5605–5615.

Cao, X., Shores, E.W., Hu-Li, J., Anver, M.R., Kelsall, B.L., Russell, S.M., Drago, J., Noguchi, M., Grinberg, A., Bloom, E.T., Paul, W.E., Katz, S.I., Love, P.E., and Leonard, W.J. (1995). Defective lymphoid development in mice lacking expression of the common cytokine receptor γ chain. Immunity *2*, 223–238.

Depper, J.M., Leonard, W.J., Drogula, C., Kronke, M., Waldmann, T.A., and Greene, W.C. (1985). Interleukin 2 (IL-2) augments transcription of the IL-2 receptor gene. Proc. Natl. Acad. Sci. USA *82*, 4230–4234.

DiSanto, J.P, Müller, W., Guy-Grand, D., Fischer, A., and Rajewsky, K. (1995). Lymphoid development in mice with a targeted deletion of the interleukin-2 receptor γ chain. Proc. Natl. Acad. Sci. USA *92*, 377–381.

Durbin, J.E., Hackenmiller, R., Simon, M.C., and Levy, D.E. (1996). Targeted disruption of the mouse *Stat1* gene results in compromised innate immunity to viral disease. Cell *84*, 443–450.

Feldman, G.M., Rosenthal, L.A., Liu, X., Hayes, M.P., Wynshaw-Boris, A., Leonard, W.J., Hennighausen, L., and Finbloom, D.S. (1997). STAT5A-deficient mice demonstrate a defect in granulocyte-macrophage colony-stimulating factor-induced proliferation and gene expression. Blood *90*, 1768–1776.

Friedmann, M.C., Migone, T.S., Russell, S.M., and Leonard, W.J. (1996). Different interleukin 2 receptor β-chain tyrosines couple to at least two signaling pathways and synergistically mediate interleukin 2-induced proliferation. Proc. Natl. Acad. Sci. USA *93*, 2077–2082.

Fujii, H., Nakagawa, Y., Schindler, U., Kawahara, A., Mori, H., Gouilleux, F., Groner, B., Ihle, J.N., Minami, Y., Miyazaki, T., et al. (1995). Activation of Stat5 by interleukin 2 requires a carboxyl-terminal region of the interleukin 2 receptor β chain but is not essential for the proliferative signal transmission. Proc. Natl. Acad. Sci. USA *92*, 5482–5486.

Gaffen, S.L., Lai, S.Y., Xu, W., Gouilleux, F., Groner, B., Goldsmith, M.A., and Greene, W.C. (1995). Signaling through the interleukin 2 receptor β chain activates a STAT-5-like DNA-binding activity. Proc. Natl. Acad. Sci. USA *92*, 7192–7196.

Gilmour, K., Pine, R., and Reich, N.C. (1995). Interleukin 2 activates STAT5 transcription factor (mammary gland factor) and specific gene expression in T lymphocytes. Proc. Natl. Acad. Sci. USA *92*, 10772–10776.

Giri, J.G., Ahdieh, M., Eisenman, J., Shanebeck, K., Grabstein, K., Kumaki, S., Namen, A., Park, L.S., Cosman, D., and Anderson, D. (1994). Utilization of the β and γ chains of the IL-2 receptor by the novel cytokine IL-15. EMBO J. *13*, 2822–2830.

Goldsmith, M.A., Lai, S.Y., Xu, W., Amaral, C., Kuczek, E.S., Parent, L.J., Mills, G.B., Tarr, K.L., Longmore, G.D., and Greene, W.C. (1995). Growth signal transduction by the human interleukin-2 receptor requires cytoplasmic tyrosines of the β chain and non-tyrosine residues of the γ_c chain. J. Biol. Chem. *270*, 21729–21737.

Horvath, C.M., and Darnell, J.E., Jr. (1997). The state of STATs: recent developments in the study of signal transduction to the nucleus. Curr. Opin. Cell Biol. *9*, 233–239.

Hou, J., Schindler, U., Henzel, W.J., Wong, S.C., and McKnight, S.L. (1995). Identification and purification of human Stat proteins activated in response to interleukin-2. Immunity *2*, 321–329.

Ihle, J.N. (1996). STATs: signal transducers and activators of transcription. Cell *84*, 331–334.

John, S., Reeves, R.B., Lin, J.-X., Child, R., Leiden, J.M., Thompson, C.B., and Leonard, W.J. (1995). Regulation of cell-type-specific interleukin-2 receptor α-chain gene expression: potential role of physical interactions between Elf-1, HMG-I(Y), and NF-κB family proteins. Mol. Cell. Biol. *15*, 1786–1796.

John, S., Robbins, C.M., and Leonard, W.J. (1996). An IL-2 response element in the human IL-2 receptor α chain promoter is a composite element that binds Stat5, Elf-1, HMG-I(Y) and a GATA family protein. EMBO J. *15*, 5627–5635.

Kaplan, M.H., Schindler, U., Smiley, S.T., and Grusby, M.J. (1996a). Stat6 is required for mediating responses to IL-4 and for development of Th2 cells. Immunity *4*, 313–319.

Kaplan, M.H., Sun, Y.L., Hoey, T., and Grusby, M.J. (1996b). Impaired IL-12 responses and enhanced development of Th2 cells in Stat4-deficient mice. Nature *382*, 174–177.

Kawabe, Y., and Ochi, A. (1991). Programmed cell death and extrathymic reduction of $V\beta8^+CD4^+$ T cells in mice tolerant to *Staphylococcus aureus* enterotoxin B. Nature *349*, 245–248.

Kneitz, B., Herrmann, T., Yonehara, S., and Schimpl, A. (1995). Normal clonal expansion but impaired Fas-mediated cell death and anergy induction in interleukin-2-deficient mice. Eur. J. Immunol. *25*, 2572–2577.

Lecine, P., Algarte, M., Rameil, P., Beadling, C., Bucher, P., Nabholz, M., and Imbert, J. (1996). Elf-1 and Stat5 bind to a critical element in a new enhancer of the human interleukin-2 receptor a gene. Mol. Cell. Biol. *16*, 6829–6840.

Leonard, W.J. (1996). STATs and cytokine specificity. Nature Med. *2*, 968–969.

Leonard, W.J., and O'Shea, J.J. (1998). Jaks and STATs: biological implications. Annu. Rev. Immunol., in press.

Leonard, W.J., Depper, J.M., Crabtree, G.R., Rudikoff, S., Pumphrey, J., Robb, R.J., Kronke, M., Svetlik, P.B., Peffer, N.J., Waldmann, T.A., et al. (1984). Molecular cloning and expression of cDNAs for the human interleukin-2 receptor. Nature *311*, 625–631.

Leonard, W.J., Noguchi, M., Russell, S.M., and McBride, O.W. (1994). The molecular basis of X-linked severe combined immunodeficiency: the role of the interleukin-2 receptor γ chain as a common γ chain, γ_c. Immunol. Rev. *138*, 61–86.

Lin, J.-X., Migone, T.S., Tsang, M., Friedmann, M., Weatherbee, J.A., Zhou, L., Yamauchi, A., Bloom, E.T., Mietz, J., John, S., et al. (1995). The role of shared receptor motifs and common Stat proteins in the generation of cytokine pleiotropy and redundancy by IL-2, IL-4, IL-7, IL-13, and IL-15. Immunity *2*, 331–339.

Lin, J.-X., Migone, T.S., Tsang, M., Friedmann, M., Weatherbee, J.A., Lin, J.-X., Mietz, J., Modi, W.S., John, S., and Leonard, W.J. (1996). Cloning of human Stat5B: reconstitution of interleukin-2-induced Stat5A and Stat5B DNA binding activity in COS-7 cells. J. Biol. Chem. *271*, 10738–10744.

Liu, X., Robinson, G.W., Gouilleux, F., Groner, B., and Hennighausen, L. (1995). Cloning and expression of Stat5 and an additional homologue (Stat5b) involved in prolactin signal transduction in mouse mammary tissue. Proc. Natl. Acad. Sci. USA *92*, 8831–8835.

Liu, X., Robinson, G.W., and Hennighausen, L. (1996). Activation of Stat5a and Stat5b by tyrosine phosphorylation is tightly linked to mammary gland differentiation. Mol. Endocrinol. *10*, 1496–1506.

Liu, X., Robinson, G.W., Wagner, K-U., Garrett, L., Wynshaw-Boris, A., and Hennighausen, L. (1997). Stat5a is mandatory for adult mammary gland development and lactogenesis. Genes Dev. *11*, 179–186.

Meraz, M.A., White, J.M., Sheehan, K.C., Bach, E.A., Rodig, S.J., Dighe, A.S., Kaplan, D.H., Riley, J.K., Greenlund, A.C., Campbell, D., et al. (1996). Targeted disruption of the Stat1 gene in mice reveals unexpected physiologic specificity in the JAK-STAT signaling pathway. Cell *84*, 431–442.

Migliorati, G., Nicoletti, I., Pagliacci, M.C., D'Adamio, L., and Riccardi, C. (1993). Interleukin-4 protect double-negative and CD4 single positive thymocytes from dexamethasone-induced apoptosis. Blood *81*, 1352–1358.

Miller, J., Malek, T.R., Leonard, W.J., Greene, W.C., Shevach, E.M., and Germain, R.N. (1985). Nucleotide sequence and expression of a mouse interleukin 2 receptor cDNA. J. Immunol. *134*, 4212–4217.

Mui, A.L., Wakao, H., Kinoshita, T., Kitamura, T., and Miyajima, A. (1995a). Suppression of interleukin-3-induced gene expression by a C-terminal truncated Stat5: role of Stat5 in proliferation. EMBO J. *15*, 2425–2433.

Mui, A.L., Wakao, H., O'Farrell, A.M., Harada, N., and Miyajima, A. (1995b). Interleukin-3, granulocyte-macrophage colony stimulating factor and interleukin-5 transduce signals through two STAT5 homologs. EMBO J. *14*, 1166–1175.

Nakamura, Y., Russell, S.M., Mess, S.A., Friedmann, M., Erdos, M., Francois, C., Jacques, Y., Adelstein, S., and Leonard, W.J. (1994). Heterodimerization of the IL-2 receptor β- and γ-chain cytoplasmic domains is required for signalling. Nature *369*, 330–333.

Negishi, I., Motoyama, N., Nakayama, K., Nakayama, K., Senju, S., Hatakeyama, S., Zhang, Q., Chan, A.C., and Loh, D.Y. (1995). Essential role for ZAP-70 in both positive and negative selection of thymocytes. Nature *376*, 435–438.

Nelson, B.H., Lord, J.D., and Greenberg, P.D. (1994). Cytoplasmic domains of the interleukin-2 receptor β and γ chains mediate the signal for T-cell proliferation. Nature *369*, 333–336.

Nosaka, T., van Deursen, J.M., Tripp, R.A., Thierfelder, W.E., Witthuhn, B.A., McMickle, A.P., Doherty, P.C., Grosveld, G.C., and Ihle, J.N. (1995). Defective lymphoid development in mice lacking Jak3. Science *270*, 800–802.

Ogasawara, J., Watanabe-Fukunaga, R., Adachi, M., Matsuzawa, A., Kasugai, T., Kitamura, Y., Itoh, N., Suda, T., and Nagata, S. (1993). Lethal effect of anti-Fas antibody in mice. Nature *364*, 806–809.

Ohbo, K., Suda, T., Hashiyama, M., Mantani, A., Ikebe, M., Miyakawa, K., Moriyama, M., Nakamura, M., Katsuki, M., Takahashi, K., et al. (1996). Modulation of hematopoiesis in mice with a truncated mutant of the interleukin-2 receptor γ chain. Blood *87*, 956–969.

Park, S.Y., Saijo, K., Takahashi, T., Osawa, M., Arase, H., Hirayama, N., Miyake, K., Nakauchi, H., Shirasawa, T., and Saito, T. (1995). Developmental defects of lymphoid cells in Jak3 kinase-deficient mice. Immunity *3*, 771–782.

Peschon, J.J., Morrissey, P.J., Grabstein, K.H., Ramsdell, F.J., Maraskovsky, E., Gliniak, B.C., Park, L.S., Ziegler, S.F., Williams, D.E., Ware, C., et al. (1994). Early lymphocyte expansion is severely impaired in interleukin 7 receptor-deficient mice. J. Exp. Med. *180*, 1955–1960.

Quelle, F.W., Wang, D., Nosaka, T., Thierfelder, W.E., Stravopodis, D., Weinstein, Y., and Ihle, J.N. (1996). Erythropoietin induces activation of Stat5 through association with specific tyrosines on the receptor that are not required for a mitogenic response. Mol. Cell. Biol. *16*, 1622–1631.

Sadlack, B., Lohler, J., Schorle, H., Klebb, G., Haber, H., Sickel, E., Noelle, R.J., and Horak, H. (1995). Generalized autoimmune disease in interleukin-2-deficient mice is triggered by an uncontrolled activation and proliferation of $CD4^+$ T cells. Eur. J. Immunol. *25*, 3053–3059.

Schindler, C., and Darnell, J.E., Jr. (1995). Transcriptional responses to polypeptide ligands: the JAK-STAT pathway. Annu. Rev. Biochem. *64*, 621–651.

Sharfe, N., Dadi, H.K., Shahar, M., and Roifman, C.M. (1997). Human

immune disorder arising from mutation of the α chain of the interleukin-2 receptor. Proc. Natl. Acad. Sci. USA *94*, 3168–3171.

Shimoda, K., van Deursen, J., Sangster, M.Y., Sarawar, S.R., Carson, R.T., Tripp, R.A., Chu, C., Quelle, F.W., Nosaka, T., Vignali, D.A., et al. (1996). Lack of IL-4-induced Th2 response and IgE class switching in mice with disrupted Stat6 gene. Nature *380*, 630–633.

Smith, K.A. (1988). Interleukin-2: inception, impact, and implications. Science *240*, 1169–1176.

Sperisen, P., Wang, S.M., Soldaini, E., Pla, M., Rusterholz, C., Bucher, P., Corthesy, P., Reichenbach, P., and Nabholz, M. (1995). Mouse interleukin-2 receptor a gene expression: interleukin-1 and interleukin-2 control transcription via distinct cis-acting elements. J. Biol. Chem. *270*, 10743–10753.

Stocklin, E., Wissler, M., Gouilleux, F., and Groner, B. (1996). Functional interactions between Stat5 and the glucocorticoid receptor. Nature *383*, 726–728.

Takeda, K., Tanaka, T., Shi, W., Matsumoto, M., Minami, M., Kashiwamura, S., Nakanishi, K., Yoshida, N., Kishimoto, T., and Akira, S. (1996). Essential role of Stat6 in IL-4 signalling. Nature *380*, 627–630.

Thierfelder, W.E., van Deursen, J.M., Yamamoto, K., Tripp, R.A., Sarawar, S.R., Carson, R.T., Sangster, M.Y., Vignali, D.A., Doherty, P.C., Grosveld, G.C., et al. (1996). Requirement for Stat4 in interleukin-12-mediated responses of natural killer and T cells. Nature *382*, 171–174.

Thomis, D.C., Gurniak, C.B., Tivol, E., Sharpe, A.H., and Berg, L.J. (1995). Defects in B lymphocyte maturation and T lymphocyte activation in mice lacking Jak3. Science *270*, 794–797.

Udy, G.B., Towers, R.P., Snell, R.G., Wilkins, R.J., Park, S.-H., Ram, P.A., Waxman, D.J., and Davey, H.W. (1997). Requirement of STAT5b for sexual dimorphism of body growth rates and liver gene expression. Proc. Natl. Acad. Sci. USA *94*, 7239–7244.

von Freeden-Jeffry, U., Vieira, P., Lucian, L.A., McNeil, T., Burdach, S.E., and Murray, R. (1995). Lymphopenia in interleukin (IL)-7 gene-deleted mice identifies IL-7 as a nonredundant cytokine. J. Exp. Med. *181*, 1519–1526.

Wakao, H., Gouilleux, F., and Groner, B. (1994). Mammary gland factor (MGF) is a novel member of the cytokine regulated transcription factor gene family and confers the prolactin response. EMBO J. *13*, 2182–2191.

Wakao, H., Harada, N., Kitamura, T., Mui, A.L., and Miyajima, A. (1995). Interleukin 2 and erythropoietin activate STAT5/MGF via distinct pathways. EMBO J. *14*, 2527–2535.

Willerford, D.M., Chen, J., Ferry, J.A., Davidson, L., Ma, A., and Alt, F.W. (1995). Interleukin-2 receptor α chain regulates the size and content of the peripheral lymphoid compartment. Immunity *3*, 521–530.

Zuniga-Pflucker, J.C., Di, J., and Lenardo, M.J. (1996). Requirement for TNF-α and IL-1 α in fetal thymocyte commitment and differentiation. Science *268*, 1906–1909.